Crystal Structure of the Worm NitFhit Rosetta Stone Protein Reveals a Nit Tetramer Binding Two Fhit Dimers

H. Pace and C. Brenner (Thomas Jefferson U.)

Abstract No. Pace1729
Beamline(s): **X8C**

Introduction: Many human cancers are initiated by loss of the *FHIT* gene which leads to defects in programmed cell death. In worms, a leading system to study cell death, Fhit is encoded as a natural fusion protein with an unrelated protein, Nit, that is a member of the nitrilase superfamily. DOE-sponsored research in David Eisenberg's group has revealed that proteins fused in one form of life that are separated in other forms of life are "Rosetta Stones" in which the fusion event decodes a previously hidden interaction between the proteins. Thus, the structure of the NitFhit Rosetta Stone protein was expected to reveal details of the interaction between Nit and Fhit in humans.

Methods and Materials: We purified NitFhit by following the nucleotide hydrolase activity of its Fhit active site, crystallized a mercury-derivatized form of the protein and solved the crystal structure of the 200 kDa tetrameric complex using two-wavelength anomalous diffraction at beamline X8C.

Results: The Nit portion of NitFhit consists of a novel α-β-β-α sandwich protein that forms a compact tetramer. The molecule assembles such that a Nit tetramer binds two Fhit dimers. The most C-terminal β-strands coded by the Nit domains are not physically located in the Nit tetramer domains but rather interact with Fhit dimer domains.

Figure 1. The Fit of Nit and Fhit: A Nit Tetramer Binds Two Fhit Dimers.

Conclusions: The NitFhit Rosetta Stone protein, the first Rosetta Stone protein with a solved 3D structure, reveals that the C-terminus of Nit has been conserved to interact with Fhit, implicating Nit in the Fhit programmed cell death pathway. The structure also reveals that Nit proteins contain an apparent enzyme active site that may prove to be a drug target for elimination of Fhit- cancer cells.

Reference: H.C. Pace, S.C. Hodawadekar, A. Draganescu, J. Huang, P. Bieganowski, Y. Pekarsky, C.M. Croce and C. Brenner, "Crystal Structure of the Worm NitFhit Rosetta Stone Protein Reveals a Nit Tetramer Binding Two Fhit Dimers," Current Biology, 10, cover + 907-917 (2000).